

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property  
Organization  
International Bureau(43) International Publication Date  
12 February 2004 (12.02.2004)

PCT

(10) International Publication Number  
**WO 2004/013658 A3**(51) International Patent Classification<sup>7</sup>: **G01N 15/14 //**  
23/04(21) International Application Number:  
PCT/EP2003/008446

(22) International Filing Date: 30 July 2003 (30.07.2003)

(25) Filing Language: English

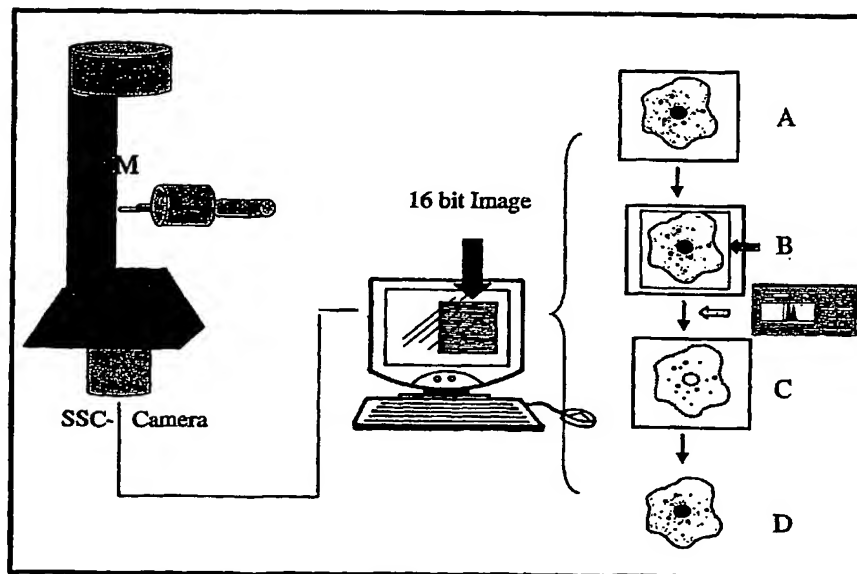
(26) Publication Language: English

(30) Priority Data:  
60/399,496 30 July 2002 (30.07.2002) US(71) Applicant (for all designated States except US): **STEIN-  
BEIS-TRANSFERZENTRUM ANALYTISCHE  
ELEKTRONENMIKROSKOPIE, BIOMEDIZIN,  
BIOTECHNOLOGIE-HEIDELBERG** [DE/DE]; Im  
Linsenhühl 21, 69221 Dossenheim (DE).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **TRENDELEN-  
BURG, Michael** [DE/DE]; Im Insenhühl 21, 69221Dossenheim (DE). **SPRING, Herbert** [DE/DE];  
Hans-Pfützner-Strasse 22a, 69198 Schriesheim (DE).  
**MONTEIRO-LEAL, Luiz Henrique** [BR/BR]; Rua  
Dona Zulmira 101/C-01, Rio de Janeiro, CEP 20550-160  
(BR). **CAMPANATI-ARAUJO, Loraine** [BR/BR]; Rua  
Rio Grande do Sul, 93/casa 11- Meier, Rio de Janeiro,  
CEP 20775-100 (BR). **TRÖSTER, Helmut** [DE/DE];  
Leibnizstrasse 4, 68165 Mannheim (DE).(74) Agent: **HARRISON, Robert**; Rouse Patents, Maximilian  
Strasse 35a, 2nd Floor, 80539 München (DE).(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,  
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,  
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,  
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,  
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,  
MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC,  
SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA,  
UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.(84) Designated States (*regional*): ARIPO patent (GH, GM,  
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),  
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),

[Continued on next page]

(54) Title: METHOD AND APPARATUS FOR MULTIPLE LABELING DETECTION AND EVALUATION OF A PLURALITY  
OF PARTICLES

(57) Abstract: The invention relates to a transmission electron microscope equipped with a 2k x 2k pixel area Slow Scan Cooled Charge Coupled Device Camera connected to an image processing software for generating an image of a sample. A segmentation of gold particles in the sample is achieved by the separation from specimen structure and background noise. An identification and classification of particle types is carried out according to the shape and size of the detected particles or particle pairs and finally, the gold particle distribution is visualized by the generation of false color overlay images as well as the indication of the numbers in the image.



European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

(88) Date of publication of the international search report:  
21 May 2004

**Published:**

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 03/08446

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 7 G01N15/14 //G01N23/04

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 7 G01N H01J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	MONTEIRO-LEAL ET AL: "Gold finder: a computer method for fast automatic double gold labeling detection, counting, and color overlay in electron microscopic images" JOURNAL OF STRUCTURAL BIOLOGY, vol. 141, - 7 February 2003 (2003-02-07) pages 228-239, XP002274390 the whole document --- -/--	1-18

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \*&\* document member of the same patent family

Date of the actual completion of the international search

22 March 2004

Date of mailing of the international search report

07/04/2004

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Bravin, M

## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/EP 03/08446

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HONG YI ET AL.: "A Novel Procedure for Pre-embedding Double Immunogold-Silver Labeling at the Ultrastructural Level " JOURNAL OF HISTOCHEMISTRY & CYTOCHEMISTRY, vol. 49, no. 3, March 2001 (2001-03), pages 279-283, XP002274391 abstract page 279, column 1, line 1 -page 280, column 1, line 25 page 281, column 1, last paragraph -page 282, column 2, line 2	1,4-7
Y	figures 2,3 ---	2,3,8-12
A	"Analyze menu" NIH IMAGE MANUAL, 'Online! 5 May 2000 (2000-05-05), XP002274392 Retrieved from the Internet: <URL:http://web.archive.org/web/20000505085723/http://rsb.info.nih.gov/nih-image/manual/menus/analyze.html> 'retrieved on 2004-03-17! page 1-2 ---	1,4-7
X	SHIMIZU H; NISHIKAWA T: "Application of an Image Analyzer to Gold Labeling in Immunoelectron Microscopy to achieve better demonstration and quantitative analysis" JOURNAL OF HISTOCHEMISTRY & CYTOCHEMISTRY, vol. 41, no. 1, 1993, pages 123-128, XP009027973	13,14,16-18
Y	abstract page 124, column 1, last paragraph -page 127, column 1, line 25 page 127, column 2, line 16-23 figures 1,2 ---	9-12,15
Y	KRIVANEK O L; MOONEY P E: "Applications of slow-scan CCD cameras in transmission electron microscopy" ULTRAMICROSCOPY, vol. 49, 1993, pages 95-108, XP009027969 the whole document ---	2,15
X	HILLER ET AL: "Performance data of a new 2048x2048 pixel slow-scan ccd camera for TEM" MICROSCOPY & MICROANALYSIS, vol. 6, no. S2, 2000, pages 732-733, XP009027049	13-18
Y	the whole document --- -/--	3,8

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/EP 03/08446

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 578 823 A (TANIGUCHI YOSHIFUMI) 26 November 1996 (1996-11-26) abstract column 8, line 59 -column 10, line 8 figure 1 ---	13-18
X	WO 01 04828 A (ELLIS BOB ;MCLAREN GINA (US); CHROMAVISION MED SYS INC (US)) 18 January 2001 (2001-01-18) abstract page 35, line 4 -page 36, line 16 page 51, line 1 -page 53, line 12 page 80, line 20 -page 90, line 13 page 100, line 4 - line 21 figure 2 figure 17A figure 22 ---	1,4-7, 9-11,13, 16-18
X	DATABASE WPI Derwent Publications Ltd., London, GB; AN 2001-172184 XP002274393 OSAME MITSUHIRO: "Double labeling detection method useful for detecting target cell or tissue, involves using chemiluminescence in situ hybridization and immune tissue chemical dyeing" & JP 2000 310637 A (UNIV KAGOSHIMA), 7 November 2000 (2000-11-07) abstract -----	13,16-18

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 03/08446

Patent document cited in search report		Publication date		Patent family member(s)	Publication date
US 5578823	A	26-11-1996	DE	19546780 A1	27-06-1996
			JP	3435949 B2	11-08-2003
			JP	8222169 A	30-08-1996
WO 0104828	A	18-01-2001	AU	6093400 A	30-01-2001
			EP	1203343 A1	08-05-2002
			JP	2003504627 T	04-02-2003
			WO	0104828 A1	18-01-2001
			US	6546123 B1	08-04-2003
JP 2000310637	A	07-11-2000	JP	3127244 B2	22-01-2001